The ability of the Aurora-A inhibitor alisertib to potentiate the anti-proliferative effects of VEGFR inhibitors in glioblastoma cells

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Abstract

Glioblastoma is the most common primary malignant brain tumor in adults, and is generally incurable. The prognosis is poor, with a median survival of only 13-16 months (Lau). Aurora-A, a serine-threonine kinase critical for a variety of cellular processes including centrosome duplication, spindle assembly, and mitotic exit, is widely overexpressed in glioblastoma. Additionally, malignant gliomas also possess high levels of vascular endothelial growth factor (VEGF), which is associated with pathological vasculogenesis and angiogenesis. Therefore, drugs that inhibit Aurora-A, in addition to VEGF and its receptor, are of interest in order to inhibit neovascularization and cellular proliferation. It was hypothesized that combining alisertib with a VEGFR inhibitor, such as cabozantinib or vandetanib, would work synergistically to inhibit glioblastoma cell proliferation and induce cell death via apoptosis. The glioblastoma cell lines U87 and U1242 were treated with a range of concentrations of alisertib and either cabozantinib or vandetanib. The results of these experiments were analyzed using Chou-Talalay and Bliss Independence models to test for synergism. Because of the lack of effective treatments available for glioblastoma, successful completion of this study may provide a basis for clinical trials including these drug combinations, and expand the potential treatment options for this aggressive disease.

Background

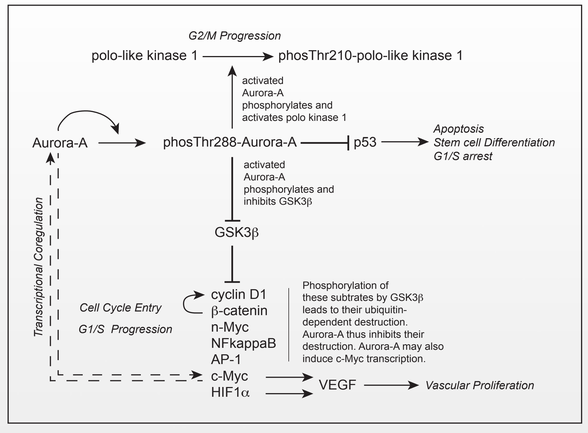


Figure 1. Pathways regulated by Aurora-A (Lehman, NL).

Background

- Both Aurora-A inhibitors and VEGFR inhibitors have been shown to possess anti-proliferative properties when used individually in glioblastoma cells (Van Brocklyn).
- In a normal brain, the expression of VEGF and its receptor is very low; however, malignant gliomas possess elevated amounts of VEGF. Additionally, Aurora-A tends to be widely overexpressed in glioblastoma. Therefore, inhibiting these two pathways is of interest in our research.
- We hypothesized that combining alisertib (MLN8237) with a VEGFR inhibitor, such as cabozantinib or vandetanib, would result in the potentiation of the anti-proliferative effects of alisertib in glioblastoma cells.

Methods

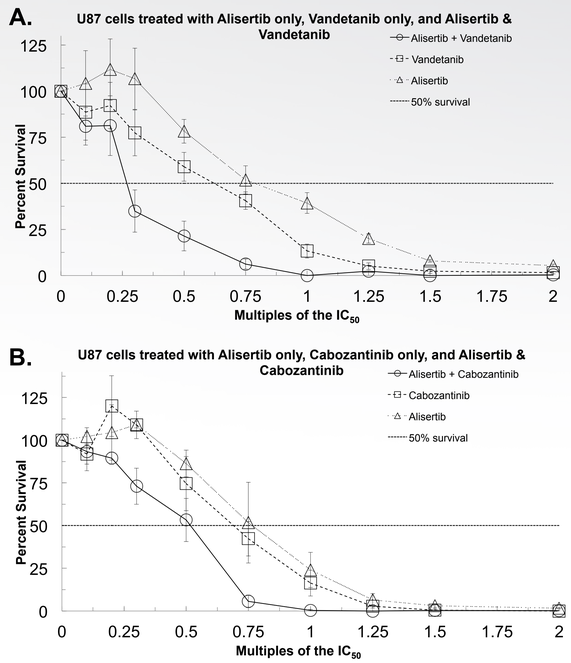
Colony Formation Assay

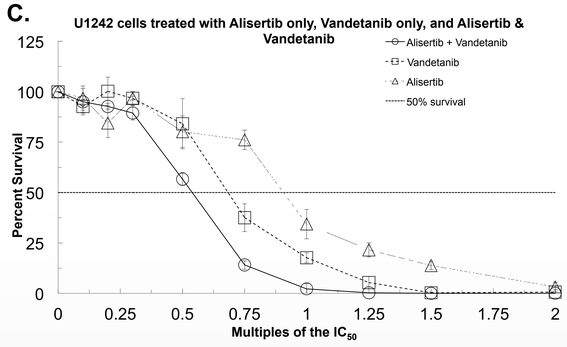
U87 and U1242 glioblastoma cells were plated at 600 cells per plate in ninety 60-mm^2 plates, then incubated for 24 hours at standard conditions. Subsequently, cells were treated in the presence of drug or sterile water corresponding to the multiples of the IC50 of each drug in each cell line; three plates were treated per condition. After three days of incubation, plates were washed in PBS to remove residual drug, and media was replaced. After an additional 3 days of incubation, plates were washed with PBS, fixed with methanol, and stained with Giemsa stain. Cells were observed and colonies counted via a dissecting microscope. Colonies containing 20 cells or more were scored. Percent survival was calculated as the number of colonies formed in treated plates/number of colonies in control untreated plates, and the results were graphed using Microsoft Excel software.

Statistical Analysis

Data expressed as percent survival of cells relative to control untreated cells was collected for each drug combination over the tested range of concentrations. Synergy is loosely defined as exhibiting more potent drug activity at lower dose levels when used in combination than when used individually at single drug doses (Zhao). The statistical methods for testing for synergism that we used include the Chou-Talalay and Bliss independence models (Bliss, Chou).

Results





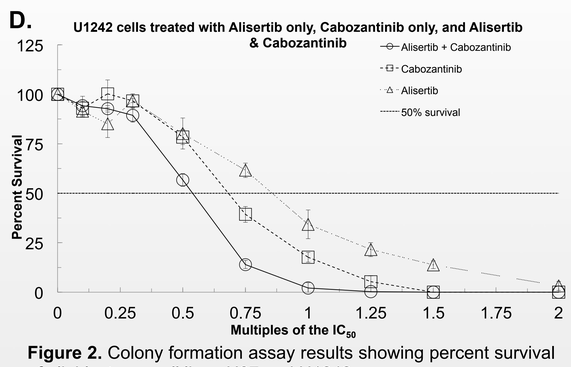


Figure 2. Colony formation assay results showing percent survival of glioblastoma cell lines U87 and U1242.

Results

- Synergism was observed predominantly in the middle dose ranges in each of the drug combinations in both cell lines.
- **Alisertib + Cabozantinib**: In the U87 cell line, synergism was exhibited in the 0.5x-1x IC50 range. In the U1242 cell line, synergism was exhibited in the 0.5x-0.75x range. Both results were observed using the Bliss independence model.
- **Alisertib + Vandetanib**: In the U87 cell line, synergism was exhibited in the 0.5x-0.75x range. In the U1242 cell line, synergism was exhibited in the 0.5x-0.75x range. Both results were observed using the Bliss independence model.

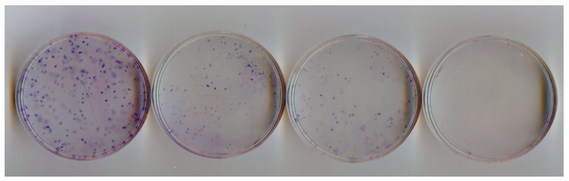


Figure 3. Colony formation assay results of U1242 cells treated with alisertib and cabozantinib. From left to right: alisertib + cabozantinib 0x, cabozantinib 1x, alisertib 1x, alisertib + cabozantinib 2x.

Conclusions

- The data suggests that alisertib has the ability to work synergistically with both cabozantinib and vandetanib in glioblastoma cell lines U1242 and U87.
- The concept that Aurora-A inhibitors used in combination with VEGFR inhibitors may be an effective glioblastoma treatment is validated by these studies at the *in vitro* level, suggesting that further *in vivo* testing in animal models and possibly clinical trials in glioblastoma patients may be warranted.

Acknowledgements

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